Brit. J. Pharmacol. (1957), **12**, 194.

OXYTOCIC ACTIVITY OF BASIC (AMINOMETHYL) DERIVATIVES OF PHENOLS AND RELATED COMPOUNDS

BY

A. COHEN, R. A. HALL, B. HEATH-BROWN, M. W. PARKES, AND A. H. REES

From the Chemical and Biological Laboratories, Research Department, Roche Products Limited, Welwyn Garden City, Herts

(RECEIVED NOVEMBER 29, 1956)

A series of piperidinomethyl and related derivatives of naphthols, substituted phenols and indoles has been tested for oxytocic activity, using *in vitro* and *in vivo* methods of assay. Some of the compounds possessed very high activity, exceeding that of ergometrine. Activity was not associated with any structural resemblance to the ergot alkaloids.

Highest activity occurred with 2-piperidinomethyl derivatives of phenols, among which maximum potency was conferred by substitution, at both the 4- and 5- positions, by methyl or ethyl or by linkage of these positions to form an indane derivative. In all series, piperidinomethyl derivatives were more active than those formed with other bases and methylation in the position α- to the nitrogen atom augmented the activity of both piperidine and morpholine derivatives. Among 2'-methyl piperidinomethyl phenols, the (−) form was more active than the (+). Acylation or alkylation of the phenolic hydroxyl group did not affect activity.

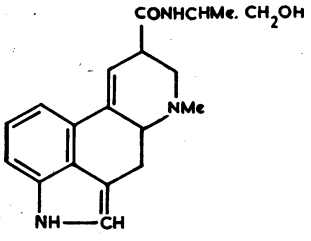
The oxytocic activity was specific, the compounds being less effective upon other forms of smooth muscle. Effects upon blood pressure and respiration of a central nature were observed.

The elaboration of synthetic drugs based on a simplification of the molecular structure of a natural prototype has been markedly successful in the fields of analgesics and curare-like compounds. Similar studies have been directed in recent years to the search for synthetic oxytocics. Much of this work has been devoted to compounds representing fragments of the structure of ergometrine (I).

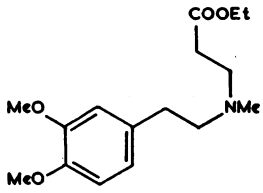
Thus Baltzly and his co-workers (Baltzly, Dvorkovitz and Phillips, 1949) reported 5 to 10% of ergometrine activity in II.

Bovet and his co-workers based their studies on the tetrahydro-2-naphthylamine fragment of ergometrine. Compounds III and IV (Marini-Bettòlo, Chiavarelli and Bovet, 1950; Marini-Bettòlo, Chiavarelli and Bovet-Nitti, 1951) were found active (Bovet-Nitti, 1952). The structures could be considerably simplified to N:N'-tetraethylglycineamide which was highly active (Bovet-Nitti, 1952). Considerable activity was also found among substituted N-phenylglycineamides (Bovet-Nitti and Bovet, 1954). Gearien and Liska (1954) found activity in V and VI, among naphthalenic structures related to ergometrine, and, to a lesser extent, in alkylated glycineamides (Rosen, Blumenthal, Townsend, Tislow and Seifter, 1956). Another active series of tetrahydro-2-naphthylamine derivatives has been reported by Kraushaar (1954). The substituted 3-phenylpiperidine derivative (VII) has also been found active (Plieninger, 1953).

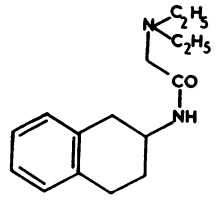
Akkerman and Veldstra (1954) and van Proosdij-Hartzema and de Jongh (1954) studied compounds of the "open-ergometrine" type (VIII), but found them far less active than the more readily prepared isomeric piperidinomethylindoles (IX) which, however, are less closely related to ergometrine (Akkerman, de Jongh and Veldstra, 1951; de Jongh and van Proosdij-Hartzema, 1952). The suggestion that oxytocic activity may not require a true partial structure of ergometrine receives further support from examples such as the simple oxytocics of Bovet (see above); in addition, significant activity has been reported for the piperidinomethylindene



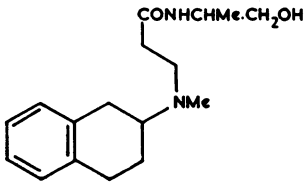
I



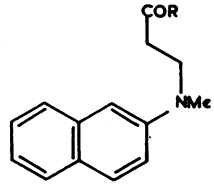
II



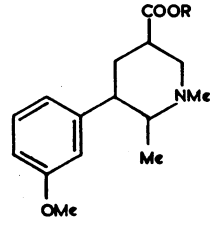
III



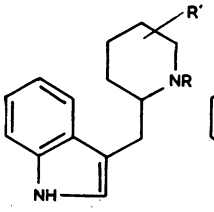
IV



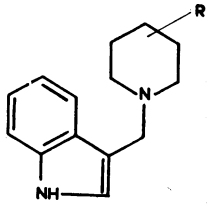
V, R=OEt
VI, R=NEt2



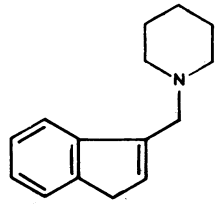
VII



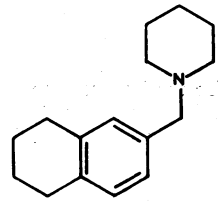
VIII



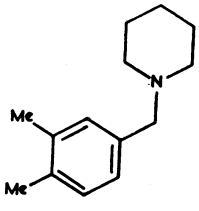
IX



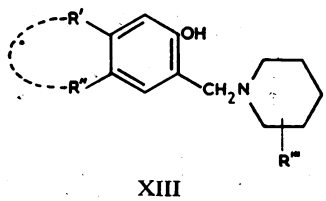
X



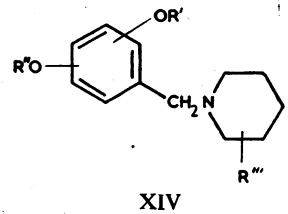
XI



XII



XIII



XIV

(X) by Hoffmann and Schellenberg (1944) and for compounds such as XI and XII by Schindler and Voegtli (1949).

It therefore appeared worth while seeking oxytocic activity outside the partial structures of ergometrine. The compounds studied in the present investigation were mainly substituted aminomethylphenols (XIII) prepared by the Mannich reaction of various secondary amines, formaldehyde and a series of phenolic substances in place of indole as used by Akkerman *et al.* (1951).

While this work was in progress information became available of the oxytocic properties of substituted N-benzylpiperidines disclosed in U.S. Patent 2589205 which included compounds closely related to or identical with a few discussed below. Another U.S. Patent, 2633468, claimed

active piperidinomethylcoumarans. More recently the activity of hydroxy- and methoxy-substituted N-benzyl piperidines (XIV) has been reported by Hejno and Arnold (1953) and Arnold and Hejno (1955).

METHODS

Isolated Guinea-pig Uterus.—Each substance was assayed by direct comparison with an oxytocic standard, usually ergometrine maleate, using a method based on that of Dale and Laidlaw for assaying pituitary extract as recommended by the *British Pharmacopoeia* (1948) and Burn (1937). One horn of the uterus from a virgin guinea-pig of 160–300 g. weight was suspended in modified Ringer solution at 36° C. containing NaCl 0.9%, KCl 0.042%, $CaCl_2$ 0.024%, $MgCl_2$ 0.044%, $NaHCO_3$ 0.05%, and dextrose 0.05%. The large quantity of magnesium chloride was adopted to reduce spontaneous activity and to sensitize the uterus to stimulation (Hsu, 1948).

Substances were assayed by a bracketing procedure, four doses constituting one comparison, comprising one dose level of test substance (T) and one dose level of standard (S), each repeated twice in a sequence STTS or TSST. Dose levels were chosen to produce contractions about 70% of maximal. Each dose was allowed to act for 3 min., when the fluid was changed and the organ rested for 6 min. before the next dose. Groups of doses were repeated, being adjusted until comparisons were obtained showing the smallest dose T producing responses greater than those due to S and also the largest dose of T which was less effective than a dose of S (Fig. 1). This gave the range within which the relative activity lay. Each assay was repeated until the range in at least two tests was narrower than ±33% of the mean value.

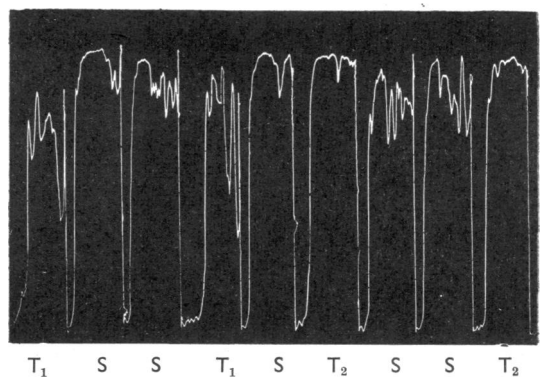


FIG. 1.—Isolated guinea-pig uterus. Bath volume 30 ml. At T_1, Ro 3–0601 0.3 mg.; at T_2, Ro 3–0601 0.5 mg.; at S, ergometrine maleate 0.01 mg. In the first set of four doses, $S > T_1$; in the second set $T_2 > S$. From this, Ro 3–0601 showed between 2 and 3% the activity of ergometrine, by weight.

Guinea-pig Uterus in situ.—Virgin guinea-pigs, weighing 200 to 600 g., were anaesthetized with urethane, 2.4 g./kg. subcutaneously, and uterine movements recorded by a modification of a method described by Bell and Robson (1936). One horn of the uterus was exposed through an abdominal incision and a silver wire hook attached to a thread was passed through the horn at its mid-point or round it by piercing the ligament below it. The thread was passed through an inverted funnel which was clamped in position over the uterus, excluding other viscera but with care not to bear on the uterus or restrict its

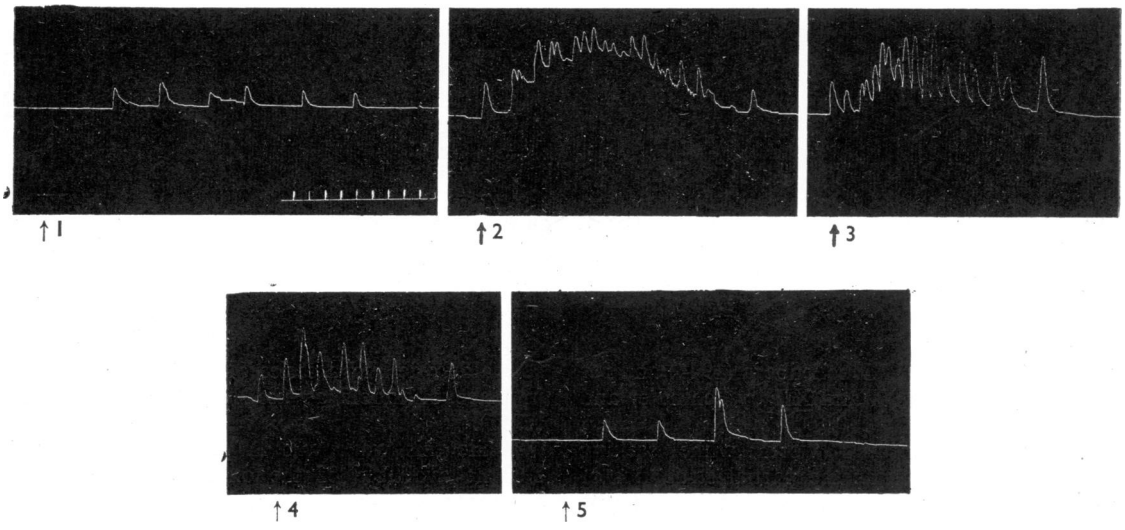


FIG. 2.—Records of the movements of the uterus *in vivo* in the guinea-pig anaesthetized with urethane. Time, 30 sec. At 1, Ro 3–0594, 2.5 μg./kg.; 2, Ro 3–0594, 5 μg./kg.; 3, Ro 3–0733, 10 μg./kg.; 4, Ro 3–0733, 5 μg./kg.; 5, Ro 3–0733, 2.5 μg./kg. From minimally effective doses (Ro 3–0594, 2.5 μg./kg.; Ro 3–0733, 2.5 μg./kg.) the two compounds were equally active.

circulation. The abdominal wall was closed round the funnel and the thread attached to a frontal lever weighted to lift the horn so that subsequent contraction exerted tension on the thread. The ends of the horn were not attached to the funnel, as in the method of Bell and Robson, so as to avoid interference with the uterus and to minimize spontaneous activity and insensitivity to stimulation. Drugs were injected into the jugular vein. Activity was measured in comparison with a standard by determining the minimally effective dose for each (Fig. 2).

Cat Uterus in situ.—Cats were anaesthetized with chloralose, 80 mg./kg. intravenously under light ether. Movements of one horn of the uterus were recorded by the method described for guinea-pigs. Recordings of blood pressure were made from the carotid or femoral artery using a mercury manometer or a membrane manometer, of heart rate using the method of Dawes (1951), and respiration by the method of Paton (1949). Drugs were injected into the saphenous vein.

Perfused Rabbit Ear and Rat Hindquarters.—Rabbits were stunned and bled. After cannulating the central artery, the ear was removed. Rats were stunned and bled. The aorta was cannulated near the iliac bifurcation and the hindquarters were separated from the remainder of the animal. Both preparations were perfused from a Mariotte bottle with modified Locke solution containing NaCl, 0.9% ; KCl, 0.042% ; $CaCl_2$, 0.024% ; $NaHCO_3$, 0.05% ; dextrose, 0.05%. Drugs were injected in small volumes through a rubber cap on a side arm near the tip of the cannula.

Bronchoconstrictor Effect. — Guinea-pigs were pithed under ether and the resistance of the bronchial tree to inflation by artificial respiration was registered by a piston recorder using the principle of Konzett and Rössler (1940). Drugs were injected into the jugular vein.

RESULTS

Oxytocic Activity in vitro.—The activity of a number of piperidinomethyl derivatives of phenolic compounds is shown in Table I. Values are expressed on a molar basis, relative to ergometrine as unity. Among the first six compounds tabulated the greatest activity was found in the 2-piperidinomethyl-4 : 5-dimethylphenol (**5**), which was twice as active as ergometrine. Analogous compounds from isomeric xylenols (**7** to **10**; **29, 30**), were all of lower activity than **5**. Additional methyl substitution in the phenolic ring generally reduced activity (**11, 12**). Among the 2 : 4 : 5-trisubstituted phenols homologous with **5** activity was unchanged on enlarging the 5-substituent to ethyl (**18**) although increase of the 4-substituent to ethyl augmented activity, both with 5-methyl (**13**) and 5-ethyl (**19**). Further enlargement of the 4-substituent to propyl or *iso*propyl (**14, 15**) diminished activity. The negligible activity of the 4-propyl compound (**16**) which is isomeric with **13** and **18** suggests that substitution of both 4- and 5-positions is necessary.

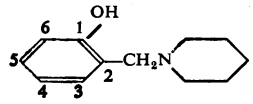
The indane derivative **21**, which also showed high activity, may be considered as containing these substituents linked to form the additional ring. No compound containing an additional 6-membered ring showed high activity.

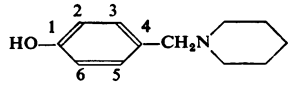
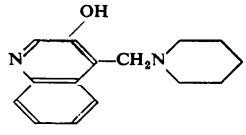
Table II shows the activities of compounds in which various basic groups are linked to 3 : 4-xylenol, 2-naphthol and indole. In the first series, the piperidine ring (3-0575) confers greater activity than does the pyrrolidine (2-6428), hexamethyleneimine ring (3-0685), or any other secondary amine. The superiority of piperidine to morpholine is seen in all three series (3-0575 and 3-0710). Linkage of the basic group to the

TABLE I

OXYTOCIC ACTIVITIES OF PIPERIDINOMETHYL DERIVATIVES OF PHENOLIC COMPOUNDS

Molar activities on isolated guinea-pig uterus (ergometrine = 1).



No.	Substituents	Activity
Ro		
1. 3–0737		0·02
2. 3–0677	5-methyl	0·03
3. 3–0676	4-methyl	0·03
4. 3–0696	4-chloro	0·03
5. 3–0575	4 : 5-dimethyl	2·0
6. 3–0698	4-chloro-5-methy	0·5
7. 3–0590	3 : 5-dimethyl	0·8
8. 3–0624	5 : 6-dimethyl	0·2
9. 3–0635	4 : 6-dimethyl	0·15
10. 3–0682	3 : 4-dimethyl	0·6
11. 3–0675	3 : 4 : 6-trimethyl	0·3
12. 3–0711	3 : 4 : 5-trimethyl	0·65
13. 3–0707	4-ethyl-5-methyl	5·5
14. 3–0734	4-propyl-5-methyl	0·6
15. 3–0777	4-*iso*propyl-5-methyl	0·3
16. 3–0748	4-propyl	<0·01
17. 3–0761	4-*cyclo*hexyl	<0·01
18. 3–0739	4-methyl-5-ethyl	2·0
19. 3–0733	4 : 5-diethyl	3·5
20. 3–0724	4 : 5-dimethoxy	0·04
21. 3–0636	4 : 5-trimethylene	1·25
22. 3–0542	4 : 5-tetrahydrobenz	0·15
23. 3–0674	4 : 5-benz	0·15
24. 3–0514	3 : 4-benz	0·3
25. 3–0533	5 : 6-benz	0·04
26. 3–0548	3 : 4-(3′ : 2′-pyrid)	0·05
27. 3–0601	3 : 4-benz-5-piperidino-methyl-6-hydroxy	0·015
28. 3–0615	3 : 4-benz-6-ethoxycarbonyl	<0·1
		
29. 3–0625	2 : 5-dimethyl	<0·05
30. 3–0634	2 : 6-dimethyl	0·02
31. 3–0524		Nil

nucleus by a single methylene carbon resulted in greater activity than when the linkage was extended (3-0742, 3-0744) or omitted (3-0820).

The effect on activity of substitution in the piperidine ring is shown in Table III for derivatives of 3:4-xylenol, 5:6:7:8-tetrahydro-2-naphthol, 2-naphthol and indole. Within the group of piperidine substituents tabulated, the 2-methyl group alone increased activity (3-0594, 3-0609, 3-0530), whereas other substitution reduced activity or had little effect on it. The advantage conferred by a 2-methyl group extended also to

TABLE II

OXYTOCIC ACTIVITIES OF BASIC DERIVATIVES OF PHENOLS, NAPHTHOLS, AND INDOLES

Molar activities on isolated guinea-pig uterus (ergometrine = 1·0).

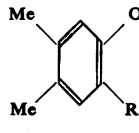
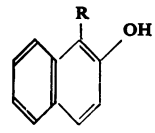
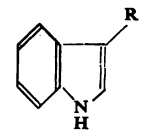
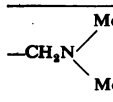
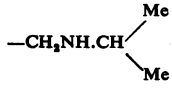
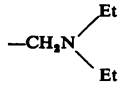
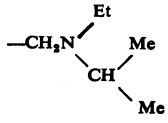
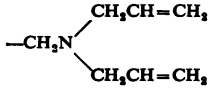
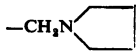
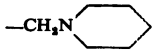
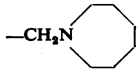
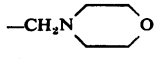
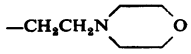
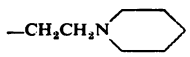
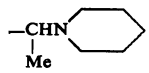
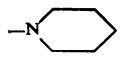
R						
	No.	Activity	No.	Activity	No.	Activity
	Ro 3–0859	0·03	Ro 3–0515	0·02	Ro	
	3–0701	0·12				
	3–0702	0·25				
	3–0699	0·25				
	3–0760	0·2				
	2–6428	0·5				
	3–0575	2·0	3–0514	0·3	3–0516	0·04
	3–0685	0·6				
	3–0710	0·1	3–0522	<0·005	3–0532	0·005
	3–0742	0·015				
	3–0744	0·3			3–0754	0·01
			3–0663	<0·02		
	3–0820	<0·05				

TABLE III

OXYTOCIC ACTIVITIES OF SUBSTITUTED PIPERIDINOMETHYL DERIVATIVES OF PHENOLS, NAPHTHOLS, AND INDOLES

Molar activities on isolated guinea-pig uterus (ergometrine = 1·0).

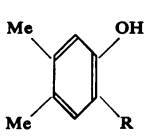
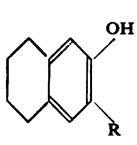
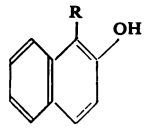
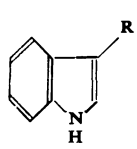
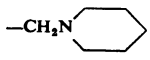
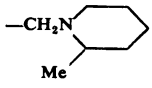
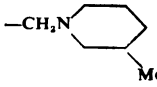
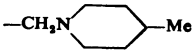
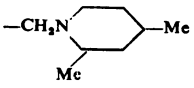
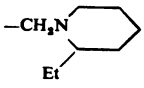
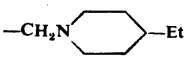
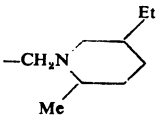
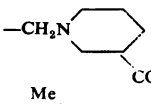
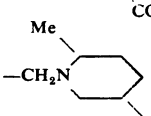
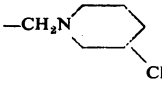
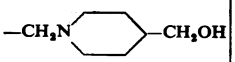
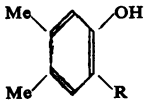
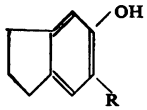
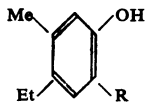
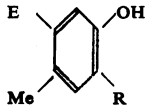
R								
	No.	Activity	No.	Activity	No.	Activity	No.	Activity
	Ro 3-0575	2·0	Ro 3-0542	0·15	Ro 3-0514	0·3	Ro 3-0516	0·04
	3-0594	4·0	3-0609	0·33	3-0540	0·12	3-0530	0·1
	3-0632	0·8			3-0612	0·1		
	3-0627	0·66			3-0541	0·1	3-0544	0·1
	3-0614	0·66			3-0545	0·15		
	3-0712	0·66						
	3-0626	0·12			3-0536	0·03	3-0537	<0·001
	3-0633	0·6						
	3-0611	<0·01	3-0610	0·2	3-0570	0·4	3-0535	Nil
	3-0722	0·2						
					3-0599	0·05		
	3-0628	0·04			3-0600	0·15		
	3-0631	0·01						

TABLE IV

EFFECT UPON OXYTOCIC ACTIVITY OF SUBSTITUTING PIPERIDINE OR MORPHOLINE GROUP BY METHYL IN α-POSITION

Molar activities (ergometrine=1·0). The *in vivo* results with cats and those marked with an asterisk were obtained by comparison with Ro 3–0594 and are expressed as molar activities relative to Ro 3–0594=4·0 (see text).

	R=—CH_2N			R=—CH_2N (Me)			R=—CH_2NO			R=—CH_2NO (Me)		
	No.	Guinea-pig	Cat	No.	Guinea-pig	Cat	No.	Guinea-pig	Cat	No.	Guinea-pig	Cat
		In vitro	*In vivo*		*In vitro*	*In vivo*		*In vitro*	*In vivo*		*In vitro*	*In vivo*
	Ro			Ro			Ro			Ro		
	3–0575	2·0	1·5	3–0594	4·0	4·0	3–0710	0·1	0·3*	3–0738	0·53	4·5*
	3–0636	1·25	0·6	3–0670	3·0	6·0*	3–0745	0·05	0·25*	3–0747	0·33	2·3
	3–0707	5·5	12·5	3–0713	13·0	3·3*						
	3–0739	2·0	3·2*	3–0752	3·0	4·0						

	3–0738	3·3
	3–0747	2·5

morpholine derivatives as seen in Table IV (3-0738), which also shows this effect in the 5-hydroxyindane series (3-0670, 3-0747).

Oxytocic Activity in vivo.—In view of the different criteria adopted in evaluating activity *in vitro* and *in vivo* in the guinea-pig, there was no reason to suppose that results from the two methods would correspond. Substances tested *in vitro* were compared at dose levels causing nearly maximal responses, while in the *in vivo* method minimally effective doses were sought. Nevertheless, Tables IV and V show that, in general, fairly close parallelism was observed. Similar agreement was also observed between *in vitro* and *in vivo* results from cat and rabbit uteri.

However, the morpholine and methylmorpholine compounds (Table IV) showed activities in both guinea-pig and cat uterus *in situ* which were consistently greater, by a factor of between 3 and 10, than those in the isolated uterus. We consider that these differences between *in vitro* and *in vivo* results indicate a reduction in relative potency at responses approaching maximal, as used in the *in vitro* test, as these compounds show less steeply inclined dose-response curves than those of the other oxytocics studied.

Owing to an inconsistency of response towards ergometrine observed in the uteri of all species tested *in vivo*, it was necessary to use another compound as a subsidiary standard and it was found convenient to test many substances against

TABLE V

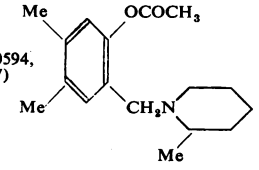
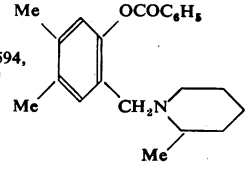
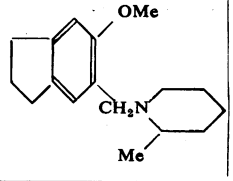
IN VITRO AND *IN VIVO* ACTIVITIES OF OXYTOCIC COMPOUNDS

Molar activities (ergometrine=1.0). The *in vivo* results marked with an asterisk were obtained by comparison with Ro 3–0594 and are expressed as molar activities relative to Ro 3–0594=4.0 (see text).

No. Ro	Guinea-pig *In vitro* (Tables I–III)	Guinea-pig *In vivo*	No. Ro	Guinea-pig *In vitro* (Tables I–III)	Guinea-pig *In vivo*
3–0514	0·3	0·6	3–0570	0·4	0·1
3–0515	0·02	0·01	3–0590	0·8	0·8
3–0516	0·04	0·07	3–0614	0·66	1·0
3–0522	<0·005	0·05	3–0632	0·8	0·8
3–0530	0·1	0·15	3–0702	0·25	0·6
3–0537	<0·001	<0·02	3–0712	0·66	0·66*
3–0540	0·12	0·6	3–0733	3·5	3·5*
3–0545	0·15	0·6	3–0734	0·6	
			3–0760	0·2	

TABLE VI

OXYTOCIC ACTIVITIES OF BASIC DERIVATIVES OF ACYLATED AND ALKYLATED PHENOLS

No.	Oxytocic Activity (Ergometrine = 1·0) Guinea-pig Uterus	
	In vitro	*In vivo*
Ro 3–0862 (cf. Ro 3–0594, Table IV) 	4·8	4·0
Ro 3–0867 (cf. Ro 3–0594, Table IV) 	3·8	2·6
Ro 3–0885 (cf. Ro 3–0670, Table IV) 	1·0	

Ro 3-0594 which had itself been standardized against ergometrine.

Effect of Modifying the Phenolic Group.—Two O-acyl derivatives, Ro 3-0862 and Ro 3-0867, and one O-methyl ether, Ro 3-0885, exerted similar oxytocic activity, *in vitro* and *in vivo*, to the parent phenols (Table VI).

They also produced effects upon the blood pressure and respiration of the chloralosed cat similar to those of the parent phenols.

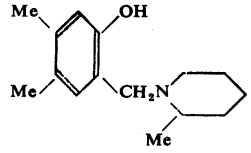
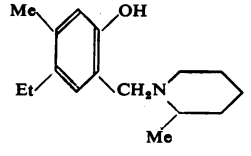
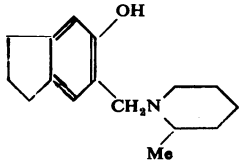
Effect of Optical Resolution.—The (−) and (+) forms of α-pipecoline were used to prepare optical isomers of three derivatives, 2-(N-2′-methylpiperidinomethyl)-4:5-dimethylphenol, 2-(N-2′-methylpiperidinomethyl)-4-ethyl-5-methylphenol and 6-(2′-methylpiperidinomethyl)-5-hydroxyindane, the racemic forms of which appear in Table IV as Ro 3-0594, Ro 3-0713 and Ro 3-0670 respectively. Table VII shows the relative activities of the (−), (+) and racemic forms of each and it may be seen that greater activity was found for the (−) forms, although the (+) forms were also considerably active.

General Pharmacology.—In contrast to our experience with ergometrine, all uteri used responded consistently to these synthetic oxytocics.

TABLE VII

OXYTOCIC ACTIVITIES OF OPTICAL ISOMERS OF α-PIPECOLINOMETHYL DERIVATIVES OF SUBSTITUTED PHENOLS

The *in vivo* results were obtained by comparison with Ro 3–0594 and are expressed as molar activities relative to Ro 3–0594 = 4·0.

No. Ro		Oxytocic Activity (Ergometrine = 1·0) Guinea-pig Uterus *In vitro*	Guinea-pig Uterus *In vivo*	Cat Uterus *In vivo*	LD50 i.v. in Mice (mg./kg.) (P = 0·05)
3–0694 	(−)	6·0	11·0	12·0	17·5 (16·2–19)
3–0679	(+)	0·8	1·6	1·6	81·0 (74·5–88·5)
3–0594	(±)	4·0	4·0	4·0	57·0 (54·5–59·5)
3–0765 	(−)	14·5	4·3	7·25	25 (21–31)
3–0771	(+)	2·3	0·85	1·1	35 (32–38)
3–0713	(±)	13·0	3·3	6·6	
3–0857 	(−)	5·2	11·0		33 (28–39)
3–0858	(+)	2·1	2·0		80
3–0670	(±)	3·0	6·0		41 (38–46)

No difference in sensitivity has been found with differences in age of animal or condition of the uterus.

The stimulant action of this class of compounds affects the uterus more than other forms of smooth muscle. Whereas isolated guinea-pig uterus contracted in response to dilutions of 1 in 10^7 of the more active compounds, isolated guinea-pig ileum and seminal vesicle required dilutions of the order of 1 in 10^5 for minimal response. Isolated rabbit ileum relaxed in response to a dilution of 1 in 10^6 (Fig. 3). The compounds were vasoconstrictors and weak antagonists of adrenaline on the perfused rabbit ear and rat hindquarters preparations (Fig. 4). They were also bronchoconstrictors in the pithed guinea-pig preparation (Fig. 5), a property shared by ergometrine.

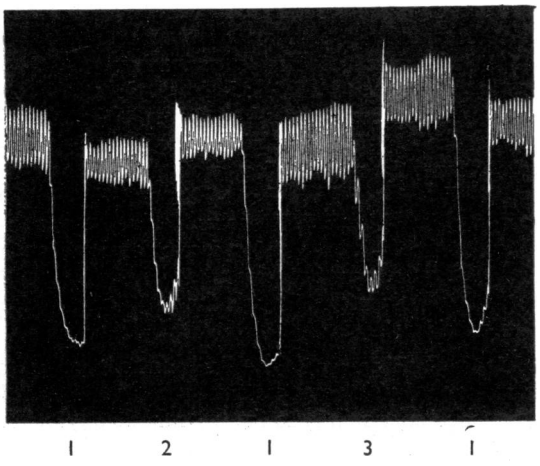


FIG. 3.—Isolated rabbit ileum. Bath volume, 30 ml. At 1, adrenaline 4 μg. 2, Ro 3-0575 30 μg. 3, Ro 3-0738 30 μg.

Lethal intravenous doses of these compounds in the mouse and rabbit caused death by respiratory arrest and convulsions ; a noticeable effect of near-lethal doses was profuse salivation. Values for LD50 by the intravenous route are given for some of the compounds in Table VIII. These values may be compared with the published LD50 for ergometrine as 145 mg./kg. (Rothlin, 1935) and 114.0 mg./kg. (Akkerman *et al.*, 1951).

On injecting the chloralosed cat intravenously with oxytocic doses of the Mannich compounds, the principal effects seen were brief respiratory arrest and fluctuation of blood pressure (Fig 6) There was also a stimulatory effect on respiration ; low doses increased rate and reduced volume, while higher doses caused rapid, shallow breathing following the initial, brief arrest.

In general, low doses of these compounds given to a chloralosed cat caused a small rise in the blood pressure which was of shorter duration than the oxytocic effect. Higher doses often produced

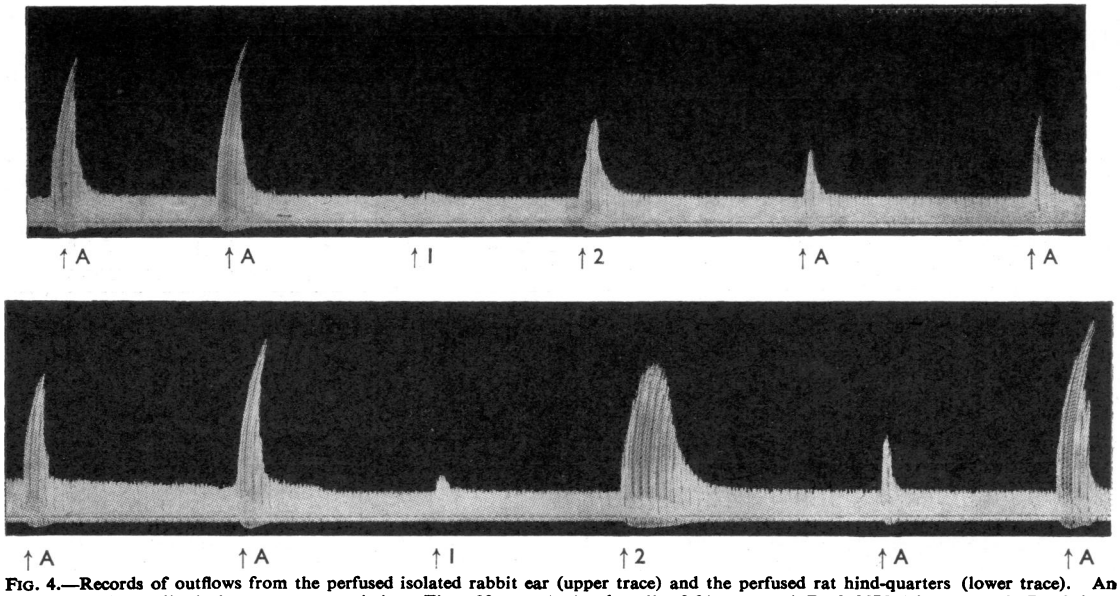


FIG. 4.—Records of outflows from the perfused isolated rabbit ear (upper trace) and the perfused rat hind-quarters (lower trace). An increase in amplitude denotes vasoconstriction. Time, 30 sec. At A, adrenaline 0.01 μg.; at 1, Ro 3–0575 1.0 mg.; at 2, Ro 3–0575 2.0 mg. The response to adrenaline was reduced after Ro 3–0575.

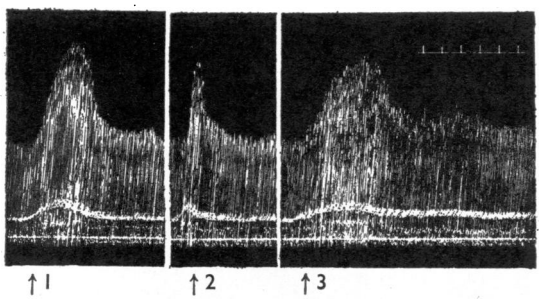


FIG. 5.—Record of bronchial resistance in the artificially respired pithed guinea-pig. A rise in bronchial tone is denoted by an increase in amplitude. Time, 30 sec. At 1, Ro 3–0575 5.0 μg./kg.; at 2, histamine 5.0 μg./kg.; at 3, Ro 3–0670 10.0 μg./kg.

TABLE VIII

LD50 VALUES FOR OXYTOCIC COMPOUNDS IN MICE (INTRAVENOUS INJECTION)

No. Ro	LD50 mg./kg. with Limits of Error in Parentheses ($P=0.05$)
3–0514	45·5
3–0536	15·0 (13·4–16·8)
3–0540	4·8 (3·8–6·05)
3–0545	44·0 (41·3–45·9)
3–0570	168·0 (150–189)
3–0575	37·0 (35–39·2)
3–0590	69·0 (61·5–77·5)
3–0614	60·0 (57·5–62)
3–0627	32·0 (27·1–36·9)
3–0632	37·0 (33–41·5)
3–0636	39·0 (34·5–44)
3–0707	56·0 (51–62)
3–0710	110·0 (103–117·5)
3–0738	118·0 (110–125)
3–0747	105·0 (94·5–116)

an initial, brief depression followed either by a rise in pressure (Fig. 6) or, usually with still larger doses, rhythmic fluctuations of as much as 60 mm. Hg, and at a rate of about 4/min., which were accompanied by similar fluctuations in respiratory movements (Fig. 7). Bradycardia was also observed. The effects upon blood pressure varied considerably in different animals and also with the anaesthetic used. Under barbiturate anaesthesia, the compounds caused only a moderately prolonged fall in blood pressure. It was notable that similar variability occurred in the response of cats to ergometrine, which was also pressor with chloralose and depressor with barbiturate anaesthesia.

Treatment with atropine, although it reduced the bradycardia, did not alter the brief depressor effect of these compounds in the chloralosed cat. This was abolished on cutting the vagi, which also prevented the effects upon respiration and further reduced the bradycardia (Fig. 7). The effect on the blood pressure was now pressor, and this was thought to be a centrally mediated action since it did not occur in the spinal cat; in this preparation depressor effects were observed with higher doses.

It is concluded that the effects upon blood pressure are due principally to two actions; a central pressor action, and a depressor effect which together with respiratory arrest and bradycardia

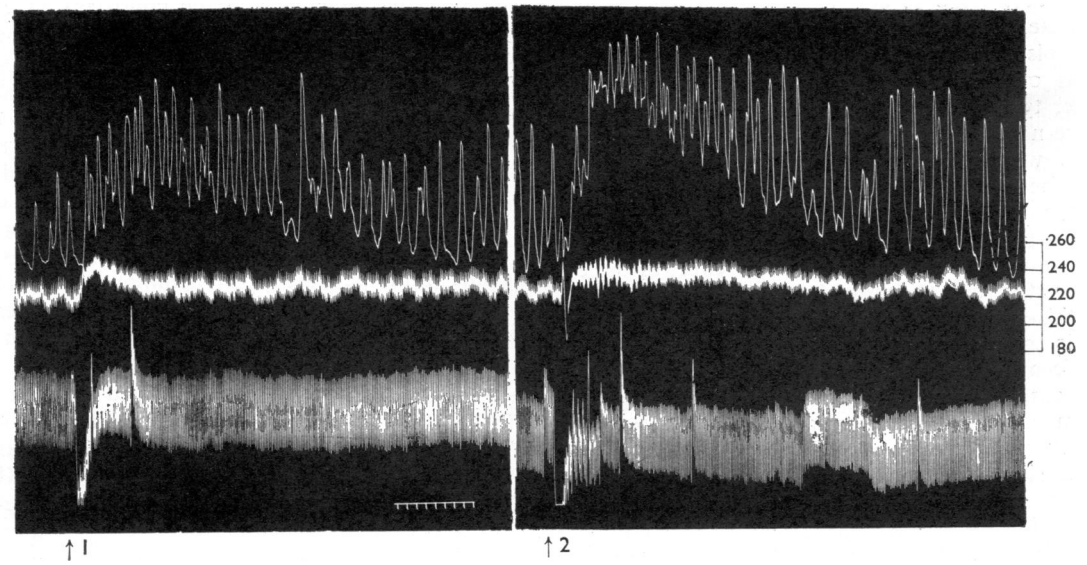


FIG. 6.—Cat anaesthetized with chloralose. The records are, from above downwards, of uterine movement, blood pressure and respiration. Time, 30 sec. At 1, Ro 3–0738, 10.0 μg./kg., and at 2, 20.0 μg./kg.

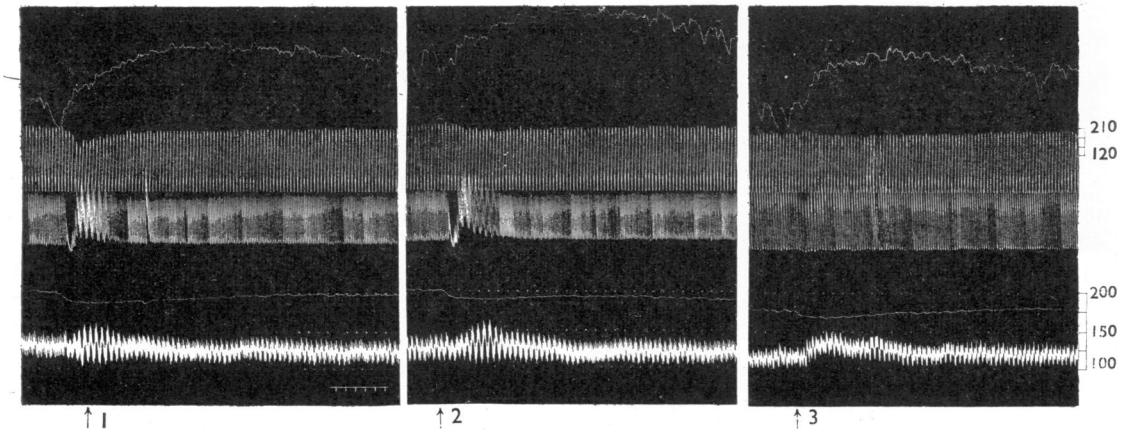


FIG. 7.—Cat anaesthetized with chloralose. The records are, from above downwards, of uterine movement, heart rate, respiration, fore-paw volume and blood pressure. Time, 30 sec. Ro 3–0707 10.0 μg./kg. at 1, 2, 3. Between 1 and 2, atropine 1.0 mg./kg., s.c. Between 2 and 3 both vagi were cut.

are mediated by the vagus. This may involve respiratory and cardiovascular reflexes resembling the Bezold reflex, similar to those excited by the veratrum alkaloids and a number of other compounds (Dawes, 1952). The resultant interaction of these effects recalls the situation found by Brown and Dale (1935) for the actions of ergometrine upon blood pressure.

DISCUSSION

Among the compounds described, many have been found to possess high oxytocic activity. The most active are several times as active as ergometrine. These basic derivatives of simple phenols have thus been shown to evoke contractions of uterine muscle more readily than any other type of synthetic compound and this is the more notable since, unlike many types studied by other workers, no structural resemblance to ergometrine can be seen in these compounds.

The simplicity of the compounds studied has enabled a series of different but closely related compounds to be examined for the effect of molecular structure upon activity. Maximum activity in this series has been shown to require, as the basic group, piperidine substituted by methyl adjacent to the nitrogen atom, and, as the nucleus, 4-methyl-5-ethyl-phenol. Divergence from this structure caused a drop in activity. The effects of changes in substituents in the basic group were similar to those described among the less active piperidinomethylindoles by Akkerman *et al.* (1951). They also found that maximum activity required a methyl substituent at either, or preferably both, of the positions adjacent to the piperidine nitrogen atom.

There is general agreement with the results of Akkerman *et al.* (1951) for the six indole derivatives Ro 3-0516, Ro 3-0530, Ro 3-0532, Ro 3-0535, Ro 3-0537 and Ro 3-0754, when our results are compared with theirs for rabbit uterus *in vivo*. However, their results for isolated guinea-pig uterus are discordant. It may be that the disagreement is due to their use of minimum effective doses as a criterion of activity *in vitro*, although our use of this criterion *in vivo* does not seem to have entailed similar discrepancies.

Uteri frequently failed to respond adequately to ergometrine *in vivo*, and the consistent effectiveness of the Mannich bases suggests that the mode of action of these compounds is not identical with that of ergometrine. Compounds based on fragments of the ergometrine molecule have not so far been found to possess more than a fraction of the oxytocic activity of the alkaloid. One molecule of the more active of the simple compounds described here, however, is as potent as 5 or more molecules of ergometrine. This, together with the absence of common structural features, supports the contention that the modes of action of these two classes of oxytocic compounds differ.

Although the compounds described here are piperidinomethyl benzene derivatives, their activity greatly exceeds that reported for the similar compounds by Schindler and Voegtli (1949) (XI and XII), and this may be attributed to the presence of an oxygen function at the *ortho*-position to the aminomethyl group. The related compounds described by Arnold and Hejno (1955) (XIII) were generally less than one-tenth as active as ergo-

TABLE IX

LIST OF COMPOUNDS

The suffixes in parentheses in column (2) refer as follows: (i) The orientation of the substituted 2-naphthols is deduced by analogy with the known 1-piperidinomethyl-2-naphthol (Caldwell and Thompson, 1939). (ii) Orientation of substituents proved by hydrogenation (Caldwell and Thompson, 1939) to 2:4:5-trimethylphenol; the orientation in the other substituted 3:4-dialkylphenols is deduced by analogy. (iii) Orientation deduced by analogy with Ro 3–0542. (iv) Orientation proved by hydrogenation to 2:3:6-trimethylphenol. (v) Structure by analogy with Ro 3–0670. (vi) Orientation proved by hydrogenation which yielded 5-hydroxy-6-methylindane, m.p. 86° to 88° C. (benzoate, m.p. 113°–114.5° C.). Fieser and Lothrop (1936) give m.p. 83°–84° C. and 111°–112° C., respectively. The letters in column 3 indicate the method of preparation given in the text of the chemical section. The numerals in parentheses in column 4 denote references as follows: (1) Auwers and Dombrowski (1906); (2) Caldwell and Thompson (1939); (3) Pohland (1949); (4) Tseou and Yang (1939); (5) Julia (1955); (6) Burckhalter, Tendick, Jones, Holcomb and Rawlins (1946); (7) Akkerman, de Jongh, and Veldstra (1951); (8) Cohen and Heath-Brown (1954a); (9) Cohen and Heath-Brown (1954b). In column 5, the names of the salts have been abbreviated in the following manner: HCl = Hydrochloride, B.HCl; M = Acid Maleate, B. $HO_2C.CH:CH.CO_2H$; T = Acid Tartrate, B.$HO_2C[CH(OH)]_2.CO_2H$.

Ro 3– (1)	Name (2)	Method of Preparation (3)	Refs. (Known Bases) Phys. Consts. °C. (New Bases) (4)	Salt with M.P. °C. (5)
0524	3-Hydroxy-4-(piperidinomethyl)quinoline	*a*	m.p. 95	
0536	1-(4′-Ethylpiperidinomethyl)-2-naphthol (i)	*a*	m.p. 113	
0540	1-(2′-Methylpiperidinomethyl)-2-naphthol	*a, b*	m.p. 94–96	
0541	1-(4′-Methylpiperidinomethyl)-2-naphthol	*a*	m.p. 131·5–133·5	
0542	3-Piperidinomethyl-5:6:7:8-tetrahydro-2-naphthol	*a, c, d*	m.p. 77–78	HCl: 197–198
0545	1-(2′:4′-Dimethylpiperidinomethyl)-2-naphthol	*a*	m.p. 71–73·5	
0548	6-Hydroxy-5-(piperidinomethyl)quinoline	*a*		HCl: 214
0570	1-(3′-Ethoxycarbonylpiperidinomethyl)-2-naphthol	*a*		HCl: 100
0575	2-Piperidinomethyl-4:5-dimethylphenol (ii)	*a*	(1)	
0590	2-Piperidinomethyl-3:5-dimethylphenol	*a*	(1, 2)	M: 121–122
0594	2-(2′-Methylpiperidinomethyl)-4:5-dimethylphenol (ii)	*a*	(3)	HCl: 190–192 M: 134–136
0599	1-(3′-Hydroxymethylpiperidinomethyl)-2-naphthol	*a*		M: 157–158
0600	1-(4′-Ethoxycarbonylpiperidinomethyl)-2-naphthol	*a*		HCl: 99–101 M: 162–163
0609	3-(2′-Methylpiperidinomethyl)-5:6:7:8-tetrahydro-2-naphthol (iii)	*a*	b.p. $120/3\times10^{-5}$ mm. n_D^{18} 1·552	T: 60–70
0610	3-(3′-Ethoxycarbonylpiperidinomethyl)-5:6:7:8-tetrahydro-2-naphthol (iii)	*a*	b.p. 180/0·3 mm.	HCl: 100 T: 75–80
0611	2-(3′-Ethoxycarbonylpiperidinomethyl)-4:5-dimethylphenol	*a*	b.p. $116/10^{-4}$ mm. n_D^{20} 1·525	
0612	1-(3′-Methylpiperidinomethyl)-2-naphthol	*a*		M: 157–158
0613	1-(2′-Methyl-5′-ethylpiperidinomethyl)-2-naphthol	*a*		M: 70
0614	2-(2′:4′-Dimethylpiperidinomethyl)-4:5-dimethylphenol	*a*	b.p. 147/0·5 mm. n_D^{20} 1·527	HCl: 180–182
0615	1-Piperidinomethyl-3-ethoxycarbonyl-2-naphthol	*a*	m.p. 106–107	M: 121–123
0624	6-Piperidinomethyl-2:3-dimethylphenol (iv)	*a*	b.p. 128–130/0·5 mm. n_D^{20} 1·537	HCl: 220–221·5
0625	4-Piperidinomethyl-2:5-dimethylphenol (ii)	*a*	(1, 2)	HCl: 226–227
0626	2-(4′-Ethylpiperidinomethyl)-4:5-dimethylphenol	*a*	m.p. 28–30	HCl: 162–164
0627	2-(4′-Methylpiperidinomethyl)-4:5-dimethylphenol	*a*	m.p. 44–46	HCl: 180–182
0628	2-(4′-Ethoxycarbonylpiperidinomethyl)-4:5-dimethylphenol	*a*	b.p. $152/5\times10^{-5}$ mm. n_D^{20} 1·522	HCl: 164–166
0631	2-(4′-Hydroxymethylpiperidinomethyl)-4:5-dimethylphenol	*a*	m.p. 75–76	HCl: 180–182
0632	2-(3′-Methylpiperidinomethyl)-4:5-dimethylphenol	*a*	m.p. 52–54	
0633	2-(2′-Methyl-5′-ethylpiperidinomethyl)-4:5-dimethylphenol	*a*	m.p. 80–81	
0634	4-Piperidinomethyl-2:6-dimethylphenol	*a*	(1)	M: 135–136
0635	2-Piperidinomethyl-4:6-dimethylphenol	*a*	(1)	M: 90
0636	6-Piperidinomethyl-5-hydroxyindane (v)	*a*	b.p. 125–126/0·22 mm. n_D^{20} 1·549 (8)	HCl: 206–208 M: 118
0663	1-(α-Piperidinoethyl)-2-naphthol	*e*		T: 125
0670	6-(2′-Methylpiperidinomethyl)-5-hydroxyindane (vi)	*a*	m.p. 35–37 (8)	HCl: 173–175 M: 152–154
0674	3-Piperidinomethyl-2-naphthol	*f*	m.p. 159–160	HCl: 217·5–219·5
0675	2-Piperidinomethyl-3:4:6-trimethylphenol	*a*	(1)	HCl: 228–230
0676	2-Piperidinomethyl-4-methylphenol	*a*	(1, 2, 4, 5)	HCl: 198
0677	2-Piperidinomethyl-5-methylphenol	*a*	(1, 2, 4, 5)	HCl: 166–168
0679	(+)-2-(2′-Methylpiperidinomethyl)-4:5-dimethylphenol	*a*	b.p. 121/0·3 mm. n_D^{20} 1·534 $[\alpha]_D^{20}$ +47·1° (c = 0·98 in benzene)	
0682	2-Piperidinomethyl-3:4-dimethylphenol	*g*	b.p. 120–122/0·18 mm. n_D^{20} 1·539	HCl: 168–170
0685	2-Hexamethyleneiminomethyl-4:5-dimethylphenol	*a*	m.p. 52	HCl: 174
0694	(−)-2-(2′-Methylpiperidinomethyl)-4:5-dimethylphenol	*a*	b.p. 112/0·14 mm. n_D^{20} 1·534 α_D^{20} −51·4° (c = 1·33 in benzene)	
0696	2-Piperidinomethyl-4-chlorophenol	*a*	(5, 6)	HCl: 231
0698	2-Piperidinomethyl-4-chloro-5-methylphenol	*a*	(6)	HCl: 207

TABLE IX—*continued*

Ro 3– (1)	Name (2)	Method of Preparation (3)	Refs. (Known Bases) Phys. Consts. °C. (New Bases) (4)	Salt with M.P. °C. (5)
0699	2-(N-ethyl-N-*iso*propylaminomethyl)-4: 5-dimethylphenol	*a*	b.p. 90/0·15 mm. n_D^{20} 1·516	HCl: 201
0701	2-Isopropylaminomethyl-4: 5-dimethylphenol	*a*	m.p. 75	HCl: 137
0702	2-Diethylaminomethyl-4: 5-dimethylphenol	*a*	(6)	HCl: 190–192
0707	2-Piperidinomethyl-4-ethyl-5-methylphenol	*a*	b.p. 120/0·1 mm. n_D^{20} 1·534	HCl: 160–162
0710	2-Morpholinomethyl-4: 5-dimethylphenol	*a*	b.p. 129/0·4 mm. (9)	HCl: 198
0711	2-Piperidinomethyl-3: 4: 5-trimethylphenol	*a*	m.p. 102–103	HCl: 211
0712	2-(2′-Ethylpiperidinomethyl)-4: 5-dimethylphenol	*a*	m.p. *ca.* 39	HCl: 174–176
0713	2-(2′-Methylpiperidinomethyl)-4-ethyl-5-methylphenol	*a*	b.p. 143/0·5 mm. n_D^{20} 1·532	HCl: 143–145
0722	2-(5′-Ethoxycarbonyl-2′-methylpiperidinomethyl)-4: 5-dimethylphenol	*a*		HCl: 189
0724	2-Piperidinomethyl-4: 5-dimethoxyphenol	*a*	b.p. 119/5 × 10^{-5} mm.	HCl: 170–172
0733	2-Piperidinomethyl-4: 5-diethylphenol	*a*	b.p. 136–138/0·1 mm.	HCl: 178
0734	2-Piperidinomethyl-5-methyl-4-propylphenol	*a*	b.p. 132/0·1 mm. n_D^{20} 1·531	
0737	2-Piperidinomethylphenol	*h*	b.p. 100/0·25 mm. n_D^{20} 1·537	HCl: 160–162
0738	2-(3′-Methylmorpholinomethyl)-4: 5-dimethylphenol	*a*	m.p. 59–61 (9)	HCl: 165–166 M: 162–164
0739	2-Piperidinomethyl-5-ethyl-4-methylphenol	*a*	b.p. 117/0·1 mm.	HCl: 154
0742	2-(β-Morpholinoethyl)-4: 5-dimethylphenol	*i*		HCl: 238–239
0744	2-(β-Piperidinoethyl)-4: 5-dimethylphenol	*j*		HCl: 193–195
0745	6-Morpholinomethyl-5-hydroxyindane (v)	*a*	m.p. 41–44 (8)	M: 133
0747	6-(3′-Methylmorpholinomethyl)-5-hydroxyindane (v)	*a*	m.p. 58–60 (8)	HCl: 193–195 M: 153
0748	2-Piperidinomethyl-4-propylphenol	*a*	b.p. 141/0·75 mm. n_D^{20} 1·528	HCl: 178–180
0752	2-(2′-Methylpiperidinomethyl)-5-ethyl-4-methylphenol	*a*	b.p. 126/0·3 mm. n_D^{20} 1·531	
0754	3-(β-Piperidinoethyl)-indole		(7)	HCl: 222.5–224·5
0760	2-Diallylaminomethyl-4: 5-dimethylphenol	*a*	b.p. 120/0·1 mm. n_D^{20} 1·529	HCl: 136–137
0761	2-Piperidinomethyl-4-cyclohexylphenol	*a*	m.p. 59–60	
0765	(−)-2-(2′-Methylpiperidinomethyl)-4-ethyl-5-methylphenol	*a*	b.p. 126 8/0·19 mm. n_D^{2} 1·530 $[\alpha]_D^{20}$ −45·7° (c = 1·25 in benzene)	
0771	(+)-2-(2′-Methylpiperidinomethyl)-4-ethyl-5-methylphenol	*a*	b.p. 126–8/0·19 mm. n_D^{20} 1·530 $[\alpha]_D^{20}$ +44·4° (c = 1·31 in benzene)	
0777	2-Piperidinomethyl-4-isopropyl-5-methylphenol	*a*	b.p. 122/0·25 mm. n_D^{20} 1·531	
0820	2-Piperidino-4: 5-dimethylphenol	*k*	b.p. 95–97/0·1 mm. n_D^{20} 1·539	
0857	(−)-6-(2′-Methylpiperidinomethyl)-5-hydroxyindane	*a*	b.p. 133–134/0·1 mm. n_D^{20} 1·549 $[\alpha]_D^{20}$ −47·2° (c = 0·68 in benzene)	M: 147–149 $[\alpha]_D^{20}$ −9·9° (c = 1·7 in water)
0858	(+)-6-(2′-Methylpiperidinomethyl)-5-hydroxyindane	*a*	b.p. 136–138/0·12 mm. n_D^{20} 1·549 $[\alpha]_D^{20}$ +44·9° (c = 1·20 in benzene)	M: 144–147 $[\alpha]_D^{20}$ +7·0° (c = 1·63 in water)
0859	2-Dimethylaminomethyl-4: 5-dimethylphenol	*a*	m.p. 80–81	
2–6428	2-Pyrrolidinomethyl-4: 5-dimethylphenol	*a*	b.p. 130/0·1 mm. n_D^{20} 1·538	HCl: 149
0862	1-Acetoxy-2-(2′-methylpiperidinomethyl)-4: 5-dimethylbenzene	*l*	b.p. 118/0·05 mm. n_D^{20} 1·527	
0867	1-Benzoyloxy-2-(2′-methylpiperidinomethyl)-4: 5-dimethylbenzene	*m*	m.p. 77–78	
0885	5-Methoxy-6-(2′-methylpiperidinomethyl)-indane	*n*	b.p. 129–131/0·05 mm. n_D^{20} 1·543	

metrine, but their figures show that the most active are also those with an oxygen function in the *ortho*-position.

Chemical Section

The known and new compounds studied were usually prepared from the appropriate phenol, base and formalin (Mannich reaction) as described in method *a*; other methods of preparation, also described below, are indicated after the name in Table IX, which also lists the properties of new bases and salts, references to literature on known bases, patent references and other details of chemical interest. Satisfactory micro-analyses were obtained for all new bases and salts. Melting points and boiling points are uncorrected.

Methods of Preparation

a.—Equivalent amounts of the phenol, base and formalin were kept overnight in alcoholic solution at room temperature, refluxed for a short period and freed from alcohol under reduced pressure. The residue was dissolved in ether, shaken with *N* sulphuric acid, and, after removal of the ether layer, the acid solution was neutralized with bicarbonate; the product was extracted with ether and isolated by crystallization or vacuum distillation. The hydrochlorides were obtained by treatment of the bases with anhydrous ethereal hydrogen chloride and crystallized from ethanol-anhydrous ether. The maleates and tartrates were obtained by treatment of the bases with molecular equivalents of the acid in alcoholic solution and precipitation with anhydrous ether; they were recrystallized from a mixture of ethanol and anhydrous ether.

b.—The phenol was refluxed in toluene with N-ethoxymethyl-pipecoline, and the product worked up as in method (*a*).

c.—1-Bromo-5 : 6 : 7 : 8-tetrahydro-2-naphthol yielded, in a Mannich reaction (method *a*), 1-bromo-3-piperidinomethyl-5 : 6 : 7 : 8-tetrahydro-2-naphthol from which bromine was eliminated by hydrogenation in acetic acid solution with a palladized barium sulphate catalyst in the presence of potassium acetate.

d.—The known 2-hydroxy-5 : 6 : 7 : 8-tetrahydro-3-naphthoic ester was converted to the *piperidide*, m.p. 202 to 204° C. (see *f*). This was reduced by lithium aluminium hydride to the product (Ro 3-0542) of known structure. This was identical with the products obtained by methods *a* and *c* from 5 : 6 : 7 : 8-tetrahydro-2-naphthol.

e.—The method of preparation was similar to *a*, but acetaldehyde was used in place of formaldehyde.

f.—By boiling 3-ethoxycarbonyl-2-naphthol with piperidine, *2-hydroxy-3-naphthopiperidide* was obtained as colourless prisms, m.p. 229 to 230° C., from methanol. Reduction with lithium aluminium hydride in ether-dioxane gave the base Ro 3-0674.

g.—2-Bromo-4 : 5-dimethylphenol yielded, in a Mannich reaction (method *a*), *2-bromo-4 : 5-dimethyl-6-piperidinomethylphenol*, m.p. 93 to 95° C. Debromination of this intermediate was effected as in method *c*.

h.—A mixture of salicylaldehyde and piperidine was hydrogenated (Pd-C catalyst) and the product worked up as in method *a*.

i.—The Kindler-Willgerodt reaction with 2-benzyloxy-4 : 5-dimethylacetophenone yielded a substituted *phenylacetothiomorpholide*, m.p. 129° C., which was desulphurized with Raney nickel. The resulting base *1-β[(2-benzyloxy-4 : 5-dimethylphenyl)ethyl] morpholine* formed a *picrate*, m.p. 178 to 178.5° C. Hydrogenation of the crude base hydrochloride with Pd-C catalyst yielded Ro 3-0742.

j.—This was prepared as in method *i* through the corresponding phenylacetothiopiperidide. The *hydrochloride* of the intermediate benzyl ether of Ro 3-0744 had m.p. 180 to 181° C.

k.—2-Amino-4 : 5-dimethylphenol reacted with 1 : 5-dibrompentane in boiling butanol in the presence of potassium carbonate. The basic product was worked up as in method *a*.

l.—Ro 3-0594 (free base) was acetylated with acetyl chloride in dry pyridine solution for 20 hr. at 20° C. After removal of solvent at reduced pressure, the basic product was obtained by treatment with cold bicarbonate solution and extraction with ether.

m.—As in *l*, using benzoyl chloride.

n.—A mixture of 5-methoxyindane-6-aldehyde and α-pipecoline was hydrogenated over palladium-charcoal, after which the basic product was isolated as in method *a*.

We wish to express our indebtedness to Dr. P. G. Philpott for the preparation of compounds Ro 3-0742, Ro 3-0744, and Ro 3-0754, included in this study; to the directors of Roche Products Limited for permission to publish these results; and to Mrs. E. B. Anderson for technical assistance.

References

Akkerman, A. M., de Jongh, D. K., and Veldstra, H. (1951). *Rec. Trav. chim. Pays-Bas*, **70**, 899.
—— and Veldstra, H. (1954). Ibid., **73**, 629.
Arnold, Z., and Hejno, K. (1955). *Chem. Listy*, **49**, 111.
Auwers, K., and Dombrowski, A. (1906). *Leibigs Ann.*, **344**, 280.
Baltzly, R., Dvorkovitz, V., and Phillips, A. P. (1949). *J. Amer. chem. Soc.*, **71**, 1162.
Bell, G. H., and Robson, J. M. (1936). *J. Physiol.*, **88**, 312.
Bovet-Nitti, F. (1952). *R.C. ist. sup. Sanit.*, **15**, 953; 989.
—— and Bovet, D. (1954). *Arch. int. Pharmacodyn.*, **96**, 327.
Brown, G. L., and Dale, H. H. (1935). *Proc. roy. Soc.*, **118***B*, 446.
Burckhalter, J. H., Tendick, F. H., Jones, E. M., Holcomb, W. F., and Rawlins, A. L. (1946). *J. Amer. chem. Soc.*, **68**, 1894.
Burn, J. H. (1937). *Biological Standardisation*, 1st ed., p. 57. London: Oxford University Press.
Caldwell, W. T., and Thompson, T. R. (1939). *J. Amer. chem. Soc.*, **61**, 2354.

Cohen, A., and Heath-Brown, B. (1954a), Brit. Pat. Appl., 28,318/54; (1954b), ibid., 29,125/54.
Dawes, G. S. (1951). *J. Physiol.*, **112**, 29P.
—— (1952). *Brit. med. Bull.*, **8**, 324.
Fieser, L. F., and Lothrop, W. C. (1936). *J. Amer. chem. Soc.*, **58**, 2050.
Gearien, J. E., and Liska, K. J. (1954). Ibid., **76**, 3554.
Hejno, K., and Arnold, Z. (1953). *Chem. Listy*, **47**, 601.
Hoffmann, K., and Schellenberg, H. (1944). *Helv. Chim. acta*, **27**, 1782.
Hsu, Y.-C. (1948). *Quart. J. Pharm.*, **21**, 146.
De Jongh, D. K., and van Proosdij-Hartzema, E. G. (1952). *J. Pharmacol.*, **105**, 130.
Julia, M. (1955). *Bull. Soc. chim. Fr.*, 830.
Konzett, H., and Rössler, R. (1940). *Arch. exp. Path. Pharmak.*, **195**, 71.
Kraushaar, A. (1954). *Arzneimittel Forsch.*, **4**, 273.
Marini-Bettòlo, G. B., Chiavarelli, S., and Bovet, D. (1950). *Gazz. chim. ital.*, **80**, 281.
—— —— and Bovet-Nitti, F. (1951). Ibid., **81**, 587.
Paton, W. D. M. (1949). *J. Physiol.*, **108**, 57P.
Plieninger, H. (1953). *Chem. Ber.*, **86**, 25.
Pohland, A. (1949). U.S. Pat. 2,589,205; *Chem. Abstr.*, 1952, **46**, 10210.
van Proosdij-Hartzema, E. G., and de Jongh, D. K. (1954). *Arch. int. Pharmacodyn.*, **98**, 335.
Rosen, H., Blumenthal, A., Townsend, P. N., Tislow, R., and Seifter, J. (1956). *J. Pharmacol.*, **117**, 488.
Rothlin, E. (1935). *C.R. Soc. Biol., Paris*, **119**, 1302.
Schindler, O., and Voegtli, W. (1949). *Pharm. Acta. Helvet*, **24**, 207.
Tseou, H.-F., and Yang, C.-T. (1939). *J. org. Chem.*, **4**, 123.